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## INFLUENCE OF SALTS ON THE NITRIC-NITROGEN ACCUMULATION IN THE SOIL.

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### INTRODUCTION

Salts which may occur in soils and those applied to them in various operations influence the number, species, and activity of the microflora of a soil. These factors are in turn reflected by the yields obtained from the soil. Some substances applied to a soil serve as food for the growing plant; others increase plant growth but not through the direct furnishing of food. This latter effect may be due to a change brought about by the salt on the physical, chemical, or bacterial properties of the soil. The substance may alter the physical properties of the soil to such an extent that the bacterial flora is modified; this in turn may increase or decrease the crop produced upon the soil. Other substances may react chemically with constituents within the soil and in so doing liberate substance which can be directly utilized by the growing plant. Again, they may directly modify the microflora and fauna of the soil both as to numbers and physiological efficiency. Or, in some cases, all three changes may be wrought by one and the same salt. The question therefore arises as to what effect this or that fertilizer or soil amendment is going to have upon the bacterial activities of the soil.

Furthermore, there are millions of acres of land in arid America which contain varying amounts of soluble salts. Some of these soils contain such large quantities of these so-called "alkalis" that no vegetation is found upon them. Other soils contain only a medium amount of soluble salts, and the vegetation is composed chiefly of alkali-resisting plants. Still other soils contain much smaller quantities of soluble salts, and they become injurious only when the soil is improperly handled. The reclaiming of the heavily charged soils and the maintaining of the others in a productive condition can be carried on successfully only when we understand the influence of salts upon the growing plants and their action upon the biological, chemical, and physical properties of the soil.

The purpose of this investigation is therefore to determine the relative toxicity of various substances found in or applied to a soil, as measured in terms of bacterial activities of the soil; also to determine the stimu-

lating influence of various substance upon bacterial activity and the manner in which the stimulation is exerted. The results thus obtained can be directly compared with those obtained for higher plants. Then, if a correlation between the two, the lower and the higher plants, be obtained, it should give a quick method of testing not only alkali soils but also other soils containing various soluble constituents. Furthermore, it makes possible the studying of balanced solutions within the soil by means of bacteriological tests, thus getting in a short time comparative results which with higher plants would be obtained only by an enormous amount of work and time. Knowledge thus obtained can be used in the reclaiming of the alkali lands of the arid West. A careful review of the literature has been made elsewhere and, hence, is not included here (3).<sup>1</sup>

#### EXPERIMENTAL WORK

The soil used in this work, taken from the College farm, is of a sedimentary nature. It was deposited by streams flowing into the valley, laden with debris derived from the near-by mountains, which are composed largely of quartzite and limestone. A physical and chemical analysis of the soil is given in Table I.

TABLE I.—Physical and chemical composition of soil

Physical composition.		Chemical composition.	
Soil.	Per cent.	Constituent.	Per cent.
Coarse sand (above 1 mm.).....	17.69	Insoluble matter.....	66.69
Fine sand (1 to 0.03 mm.).....	37.39	Potash ( $K_2O$ ).....	.55
Coarse silt (0.03 to 0.01 mm.).....	15.19	Soda ( $Na_2O$ ).....	.49
Medium silt (0.01 to 0.003 mm.).....	10.36	Lime ( $CaO$ ).....	7.41
Fine silt (0.003 to 0.001 mm.).....	10.32	Magnesia ( $MgO$ ).....	4.15
Clay (below 0.001 mm.).....	9.05	Ferric oxid ( $Fe_2O_3$ ).....	2.93
Moisture and loss.....		Alumina ( $Al_2O_3$ ).....	3.49
		Phosphorus pentoxid ( $P_2O_5$ ).....	.25
		Sulphur trioxid ( $SO_3$ ).....	.07
		Carbon dioxid ( $CO_2$ ).....	7.62
		Humus.....	2.18
		Total nitrogen.....	.15

The soil used, therefore, was a sandy loam very high in acid-soluble constituents, but the water-soluble constituents were not excessive. The calcium and magnesium contents were very high and mainly in the form of the carbonate. The soil was well supplied with phosphorus and potassium; and there was a fairly large quantity of iron present. In fact, all of the elements of plant food were present in abundance, with the exception of nitrogen, which was low. The soil was very productive, and previous work had shown the ammonifying and nitrifying powers of the soil to be about the average for the soils of the arid regions. The

<sup>1</sup> Reference is made by number (italic) to "Literature cited," pp. 134-135.

nitrogen-fixing powers of the soil were above the average, and previous work had shown it to have an intensely interesting bacterial flora.

Several hundred pounds of the soil were thoroughly mixed, stored in a large box, and kept as near field conditions as possible so that all the work could be done on the same soil. As the soil was needed in the work, portions were brought to the laboratory, air-dried in the dark, then weighed in 100-gm. portions into sterile covered tumblers. To each of these were added 2 gm. of dried blood. The whole was then carefully mixed, and the salt in most cases added from a carefully standardized stock solution. This, together with sufficient sterile distilled water to make the moisture content up to 20 per cent, was thoroughly mixed in the soil. Each series, together with sterile blanks, was incubated at 28° to 30° C. for 21 days, and then the nitric nitrogen determined as follows (*4*, p. 200):

The contents of the beaker, together with 500 cc. of distilled water and 2 gm. of alum, were placed in quart Mason jars and agitated for five minutes in a shaker.

An aliquot part (100 cc.) of the supernatant liquid was pipetted off, and, together with 2 cc. of a saturated solution of sodium hydroxid, was evaporated to about one-fourth of its original volume to free it from ammonia. To this were added 50 cc. of ammonia-free water, 5 gm. of "iron-by-hydrogen" and 30 cc. of sulphuric acid (sp. gr. 1.35). If less than 40 mgm. of nitric nitrogen is to be determined, it is well to take a correspondingly smaller quantity of iron and sulphuric acid. The neck of the reduction flask was fitted with a 2-hole stopper, through which passed a 50-cc. separatory funnel and a bent tube which dipped into a vessel containing water in order to prevent mechanical loss. The acid was slowly added and allowed to stand until the rapid evolution of hydrogen was over. It was then heated to boiling for 10 minutes. The contents of the side vessel were returned to the reduction flask before the reaction was complete, thus insuring the complete reduction of any nitrates which may have been carried over with the first violent evolution of the hydrogen. The contents of the reduction flask were transferred to Kjeldahl flasks, neutralized with sodium hydroxid, and distilled into standard acid. The excess of acid was titrated back with standard alkali, lacmoid being used as an indicator; controls were made on all the reagents, including the alum used as a flocculant.

In every case at least four determinations were made with each concentration of the salt, and, in the absence of agreement between determinations, the series was repeated so that the results as herein reported are in every case the average of four or more closely agreeing determinations. Hence, experimental error has been reduced to as near a minimum as possible in this kind of work.

The solutions of the salts were prepared by weighing gram-molecular quantities of Merck's best grade of the respective salts into 1,000 cc. of

sterile distilled water and then quantitatively determining the amount present. In those cases in which the analysis showed the concentration wrong, it was corrected, so that we have a definite knowledge of the quantity of salt added to the soil, as the varying results reported by different investigators can in many cases be interpreted by the unknown variation in salts added. The solution thus prepared was then added to the soil in such quantities as to make the amount of the anion and of cation the same and directly comparable the one with the other. The comparatively insoluble salts, calcium carbonate, calcium sulphate, etc., were carefully weighed and intimately mixed with the soil. The arranging of the work in this order gives us as nearly absolute results as can be obtained by the present bacteriological methods, and at the same time gives us directly comparable results, which after all is what we have to look for in this work.

The salts tested were the chlorids, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron.

#### INFLUENCE OF SODIUM SALTS

The compounds used in this series were sodium chlorid, sodium sulphate, sodium nitrate, and sodium carbonate. They were in concentrations such that equivalent quantities of sodium in the various forms could be directly compared. The strengths varied from 0 to 1,380 p. p. m. of soil, and represented the actual proportion of sodium in the various forms applied to the soil. The results are reported as percentage, considering the nitric nitrogen produced in the untreated soil in each case as 100 per cent. This method of reporting the results makes them more directly comparable than if stated as milligrams of nitric nitrogen formed in 100 gm. of soil. The average nitrifying power of the untreated soil was 53 mgm. of nitric nitrogen per 100 gm. of soil. The results are given in Table II, and in every case are the average of at least four and sometimes of several times this number of closely agreeing determinations; hence, they should represent very closely the comparative influence of the various sodium salts upon nitrification.

Sodium chlorid is the only one of the sodium salts tested which increases the accumulation of nitric nitrogen in the soil. In this regard nitrification differs widely from ammonification, for in the latter both sodium nitrate and sodium carbonate stimulate. However, sodium chlorid is a much more active stimulant of the nitrifiers than it is of the ammonifiers, and it stimulates in much higher concentration, being the most active when the soil contains 230 p. p. m., and is not toxic until the quantity in the soil exceeds 460 p. p. m. The toxicity rapidly increases above this concentration, and at a concentration of 1,380 p. p. m., the nitric nitrogen present had been reduced to 16.4 per cent of what it was in the original soil.

TABLE II.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of sodium salts

[The untreated soil is taken as 100 per cent]

Amount of sodium. <i>P. p. m.</i>	Percentage of nitric nitrogen formed in presence of —			
	Sodium chlorid.	Sodium sulphate.	Sodium nitrate.	Sodium carbonate.
None.....	100.0	100.0	100.0	100.0
3.6.....	102.4	87.8	92.7	100.0
7.2.....	102.5	60.3	88.5	70.4
14.4.....	100.6	57.1	94.4	70.9
28.8.....	105.1	86.2	101.0	88.1
57.5.....	114.7	74.0	75.9	94.1
115.0.....	139.6	55.1	71.6	79.4
230.0.....	142.0	65.4	69.4	73.5
460.0.....	136.2	63.2	48.0	76.5
920.0.....	57.5	63.0	17.1	63.5
1,380.0.....	16.4	50.8	— 14.0	58.8

Although sodium carbonate is toxic in the lowest concentration tested, yet its toxicity does not increase as rapidly as does that of the chlorid, for at the highest concentration it still produced 58.8 per cent of nitric nitrogen. The action of sulphate and carbonate nearly parallel each other throughout the entire series.

Sodium nitrate probably stimulates slightly at 28.8 p. p. m., but above this concentration the nitric nitrogen rapidly decreases and when the concentration of sodium in the form of sodium nitrate reaches 1,380 p. p. m., there is an actual loss of nitric nitrogen from the soil.

It is quite evident from these results that the order of toxicity of these salts are as follows: Sodium sulphate, sodium carbonate, sodium nitrate, and sodium chlorid; but if we consider them at the highest concentrations the order becomes sodium nitrate, sodium chlorid, sodium sulphate, and sodium carbonate.

The results for sodium chlorid confirm the findings of C. B. Lipman (8) as opposed to Dehérain (1) that sodium chlorid does stimulate the nitrifying bacteria. Although sodium carbonate is toxic in comparatively low concentrations, it is not as toxic in this soil as it was found to be in the soil used by Lipman; moreover, he noted a stimulation with sodium sulphate which does not appear in any of our work. This apparent discrepancy must be due to a difference in the soils used.

At the lower concentrations a given molecular quantity of sodium sulphate is more toxic to the nitrifying organisms than is an equivalent molecular proportion of sodium carbonate, and this is more toxic than is an equivalent molecular proportion of sodium nitrate. Sodium chlorid is less toxic than any of the other salts tested. When  $4 \times 10^{-4}$  mole of sodium chlorid, sodium sulphate, or sodium carbonate are added to 100 gm. of soil, the nitrate accumulation is reduced to about one-half

normal. But when an equivalent quantity of sodium in the form of the nitrate is added, the nitrifying powers are reduced to one-fifth.

The work was so planned that equal molecular proportions of the various salts could be compared as shown in Table III.

TABLE III. —Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of sodium salts in equal molecular proportions

[The untreated soil is taken as 100 per cent]

Proportion of molecular weight in 100 gm. of soil	Percentage of nitric nitrogen formed in presence of—			
	Sodium chlorid.	Sodium sulphate.	Sodium nitrate.	Sodium carbonate.
None	100.0	100.0	100.0	100.0
$156 \times 10^{-3}$	102.4	60.2	92.7	79.4
$312 \times 10^{-3}$	102.5	57.1	88.3	76.0
$625 \times 10^{-3}$	100.6	86.2	94.4	88.2
$125 \times 10^{-2}$	103.1	74.0	101.0	94.1
$25 \times 10^{-1}$	114.7	55.1	75.9	79.4
$5 \times 10^0$	139.6	65.4	71.6	73.5
$1 \times 10^{-1}$	142.7	63.2	69.4	76.5
$2 \times 10^{-2}$	136.2	63.0	48.0	58.8
$4 \times 10^{-3}$	57.5	50.8	17.1	58.8

Sodium chlorid, which has the lowest molecular weight, is the least toxic, whereas sodium nitrate, with the next lowest, comes next in order of toxicity. It is, therefore, quite evident that the toxicity of the sodium salts varies with the electro-negative ion with which sodium is combined and must be due to a physiological influence exerted by it upon the protoplasm of the organisms and not due to a direct osmotic effect.

#### INFLUENCE OF POTASSIUM SALTS

The compounds used in the potassium series were the chlorid, sulphate, nitrate, and carbonate. The concentrations were the same as those used in the sodium series. The results, as reported in Table IV, are the average of four or more closely agreeing determinations.

Potassium chlorid and potassium nitrate are the only potassium salts which yielded a stimulation with the nitrifying organisms. But after the stimulating concentration is exceeded these compounds rapidly increase in toxicity, so that by the time a concentration of 2,346 p. p. m. of potassium in the form of the chlorid has been added to the soil, the nitric nitrogen present had been reduced to 5.23 per cent. Where a similar quantity of the nitrate was added, there was a marked loss of nitrates. • •

Potassium sulphate was found to be toxic in the lowest concentration tested, but the toxicity does not increase as rapidly with the increased concentrations of this salt as it does with those salts which are stimulants. For it is only when 1,564 p. p. m. of potassium in the form of potassium sulphate has been added to the soil that we find the nitric-nitrogen accumulation reduced to one-half normal.

TABLE IV.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and different forms of potassium

[The untreated soil is taken as 100 per cent]

Amount of potassium.  <i>P. p. m.</i>	Percentage of nitric nitrogen formed in presence of —			
	Potassium chlorid.	Potassium sulphate	Potassium nitrate.	Potassium carbonate
None.....	100.0	100.0	100.0	100.0
6.1.....	93.5	79.7	100.4	99.1
12.2.....	86.8	95.7	74.7	92.7
24.4.....	106.5	95.0	98.6	81.3
48.8.....	102.3	93.8	92.5	80.3
97.8.....	84.3	73.4	100.5	69.0
195.5.....	84.3	76.4	71.1	70.9
391.0.....	84.8	67.2	33.5	63.7
782.0.....	78.0	62.3	20.0	55.4
1,564.0.....	35.6	51.5	2.5	41.8
2,346.0.....	5.2	32.4	41.2	14.8

Potassium carbonate in the lower concentrations is less injurious than is the sulphate, but in higher concentrations it becomes much more toxic.

It may be seen that the action of the chlorid and carbonate are very similar, and one must conclude that the anion, in addition to the cation, exerts a very marked effect upon the nitrifying organisms of the soil. The nitrifying powers of the soil are reduced to about half-normal with nearly the same concentration of potassium in the form of the chlorid, sulphate, and carbonate, whereas the nitrate reduces it to half-normal when one-fourth the concentration has been reached.

The influence of equivalent molecular proportions on the various potassium salts is given in Table V.

TABLE V.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of potassium salts in equal molecular proportions

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Percentage of nitric nitrogen formed in presence of —			
	Potassium chlorid.	Potassium sulphate.	Potassium nitrate.	Potassium carbonate.
None.....	100.0	100.0	100.0	100.0
$156 \times 10^{-2}$ .....	93.5	95.7	106.4	92.7
$312 \times 10^{-2}$ .....	86.8	95.0	74.7	81.3
$625 \times 10^{-2}$ .....	106.5	93.8	98.6	80.3
$125 \times 10^{-3}$ .....	102.3	73.4	92.5	69.0
$25 \times 10^{-3}$ .....	84.3	76.4	100.5	71.0
$5 \times 10^{-3}$ .....	84.3	67.2	71.1	63.7
$1 \times 10^{-3}$ .....	84.8	62.3	33.5	55.4
$2 \times 10^{-3}$ .....	78.0	51.5	20.0	41.8
$4 \times 10^{-3}$ .....	35.6	32.4	2.5	14.8



In the lower concentrations a given molecular quantity of potassium carbonate is much more toxic to the nitrifiers than is an equivalent molecular quantity of the sulphate, nitrate, or chlorid, the toxicity of the compounds varying in the order named.

In the highest concentration tested the chlorids reduce the nitrifying powers to 35.6 per cent, the sulphate to 32.4 per cent, the nitrate to 2.5 per cent, the carbonate to 14.8 per cent.

The toxicity of the potassium salts is governed largely by the electro-negative ion combined with the potassium and if the osmotic pressure plays any great part it is masked by other factors.

#### INFLUENCE OF CALCIUM SALTS

The compounds used in this series were calcium chlorid, calcium nitrate, calcium sulphate, and calcium carbonate. The first two were added to the soil according to the usual method from a standard solution, whereas the sulphate and carbonate were weighed into the soil, carefully mixed, and then treated in the ordinary manner.

A number of determinations were made in each case and compared with sterile blanks, so that the results as reported in Table VI are the average of four or more closely agreeing determinations.

TABLE VI.—Percentage of nitric-nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of calcium salts

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil	Amount of calcium.	Percentage of nitric-nitrogen formed in presence of—			
		Calcium chlorid.	Calcium sulphate.	Calcium nitrate.	Calcium carbonate.
None.....	P. p. m. None	100.0	100.0	100.0	100.0
$78 \times 10^{-2}$ .....	3.12	87.9	117.1	99.1	99.4
$156 \times 10^{-2}$ .....	6.24	79.0	115.9	88.8	97.2
$112 \times 10^{-2}$ .....	12.35	88.2	143.9	92.5	97.2
$625 \times 10^{-2}$ .....	24.82	84.0	140.9	102.1	85.3
$125 \times 10^{-1}$ .....	40.64	86.8	125.0	92.1	95.9
$25 \times 10^{-1}$ .....	99.28	127.4	148.8	100.8	97.2
$5 \times 10^{-1}$ .....	198.56	160.3	151.7	86.9	79.0
$1 \times 10^{-1}$ .....	397.12	167.6	180.6	46.8	82.0
$2 \times 10^{-2}$ .....	794.24	124.5	196.7	— 0.4	59.2
$3 \times 10^{-2}$ .....	1191.36	99.2	189.2	— 20.2	59.1

In marked counterdistinction to its action on the ammonifying organisms, calcium carbonate fails to stimulate in any of the concentrations. This, however, is not surprising, as the soil which is being used in this work contains over 12 per cent of calcium carbonate, which is undoubtedly abundant for the maximum activity of these organisms. We do, however, find a gradual increase in toxicity as the quantity of calcium carbonate added to the soil increases, so that by the time 1,191.4 parts of calcium in the form of the carbonate have been added the nitrifying

fyng powers of the soil have been reduced to 59 per cent of normal, thus indicating that it is possible to add sufficient limestone to a soil to reduce its nitric-nitrogen content. Whether this be due to its direct action upon the bacterial activities or to changing of the calcium : magnesium-carbonate ratio of the soil can not be answered by these results. It is certain it can not be due to the anions alone, for we find the other calcium salts acting as stimulants.

Calcium nitrate stimulates only slightly, and this at the medium concentrations. At low concentrations it is not as toxic as is calcium carbonate, but at concentrations of 397.1 p. p. m. and above of calcium in the form of calcium nitrate it is highly toxic. At the highest concentration tested, 1,191.4 p. p. m., the nitric nitrogen rapidly disappeared from the soil.

Calcium sulphate stimulated in all of the concentrations tested, and in most cases there was a very marked increase in the nitric nitrogen of the soil. This undoubtedly accounts for the increased plant growth noted when gypsum is added to a soil. When 794.2 p. p. m. of calcium in the form of the sulphate had been added to the soil, there was nearly twice as much nitric nitrogen in the treated soil as there was in the untreated soil.

Calcium chlorid is apparently toxic at the lower concentrations, but at the higher concentrations it becomes a marked soil stimulant. Its highest stimulation is noted at a concentration slightly lower than that of calcium sulphate. Furthermore, it becomes toxic again, a condition which has not been observed for the sulphate.

It may be noted that  $3 \times 10^{-3}$  mole of calcium nitrate reduces the nitric-nitrogen content of the soil 120 per cent below normal, while an equivalent quantity of the carbonate reduces it 59.1 per cent below normal. The chlorid at this concentration is without effect, whereas the sulphate is a very strong stimulant.

#### INFLUENCE OF MAGNESIUM SALTS

The compounds used in the magnesium series were the chlorid, sulphate, nitrate, and carbonate of magnesium. The last-named was applied to the soil in the form of a dry powder, whereas all of the others were added in the usual manner. The results representing the average of four or more closely agreeing determinations are given in Table VII.

Both the nitrate and carbonate of magnesium increase the nitric-nitrogen content of the soil. In the case of the carbonate this is very marked and is markedly different from the results obtained with calcium carbonate.

The nitrate, after reaching its highest stimulating point, rapidly becomes toxic, so that by the time 729.6 p. p. m. of magnesium in the form of the nitrate had been added to the soil, there was a loss of 354.7 per cent of the nitrogen.

TABLE VII.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of magnesium salts

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Amount of magnesium.	Percentage of nitric nitrogen formed in presence of—			
		Magnesium chlorid.	Magnesium sulphate.	Magnesium nitrate.	Magnesium carbonate.
None	P. p. m. None	100.0	100.0	100.0	100.0
$78 \times 10^{-7}$	1.9	67.7	95.2	92.9	90.4
$156 \times 10^{-7}$	3.8	58.0	90.2	85.7	97.7
$312 \times 10^{-7}$	7.6	68.5	101.2	106.5	108.1
$625 \times 10^{-7}$	15.2	55.0	87.1	87.0	136.1
$125 \times 10^{-6}$	30.4	97.1	88.7	95.7	119.8
$25 \times 10^{-5}$	60.8	123.2	94.6	65.2	140.7
$5 \times 10^{-4}$	121.6	84.5	90.4	43.5	101.2
$1 \times 10^{-3}$	243.2	78.6	82.9	.....	116.0
$2 \times 10^{-3}$	486.4	26.5	63.3	5.0	72.7
$3 \times 10^{-3}$	729.6	11.8	59.6	-354.7	51.7

Magnesium carbonate reaches its highest stimulation at a concentration of  $25 \times 10^{-5}$  mole, and at a concentration of  $3 \times 10^{-3}$  mole the ammonifying powers had been reduced to one-half normal almost the same as was the case with the calcium carbonate.

It is indeed interesting to note that this soil, which already contains over 8 per cent of magnesium carbonate, has its nitrifying powers increased by the addition of magnesium carbonate. This would lend support to the idea promulgated in the last section that the depressing effect of the calcium carbonate is due to its changing of the lime-magnesia ratio in the soil.

All of the magnesium salts are peculiar in that at the lower concentrations they are toxic, but that at higher concentrations they are stimulants. This peculiarity is especially noticeable in the case of magnesium chlorid.

Both the chlorid and sulphate were toxic in all but one of the concentrations tested. The sulphate and carbonate of magnesium at the highest concentration tested reduced the nitrifying powers to about one-half normal, while the same concentration of the chlorid reduced it to less than one-eighth normal. A similar concentration of the nitrate caused a rapid disappearance of the nitrate from the soil.

It is quite evident from these results that the determining factor in the action of calcium or magnesium salts upon the nitrifying powers of the soil is the electro-negative ion.

#### INFLUENCE OF MANGANESE SALTS

The compounds used in this series were manganous chlorid, manganous sulphate, manganous nitrate, and manganous carbonate. The results so obtained as the average of a great number of closely agreeing determinations are given in Table VIII.

TABLE VIII.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of manganese salts

[The untreated soil is taken as 100 per cent.]

Fraction of molecular weight in 100 gm. of soil.	Amount of manganese	Percentage of nitric nitrogen formed in presence of—			
		Manganous chlorid.	Manganous sulphate.	Manganous nitrate	Manganous carbonate.
None.....	P. p. m. None.	100.0	100.0	100.0	100.0
$78 \times 10^{-2}$ .....	4.3	100.0	113.2	121.3	91.2
$156 \times 10^{-2}$ .....	8.6	112.9	100.0	113.8	72.2
$312 \times 10^{-2}$ .....	17.2	57.7	100.0	113.5	77.8
$625 \times 10^{-2}$ .....	34.4	41.7	100.0	125.4	86.3
$125 \times 10^{-1}$ .....	68.8	31.9	104.1	107.9	108.4
$25 \times 10^{-1}$ .....	137.6	44.2	97.6	79.4	86.8
$5 \times 10^{-1}$ .....	275.2	55.2	101.3	49.2	84.6
$1 \times 10^{-1}$ .....	550.4	55.2	88.7	26.9	84.6
$2 \times 10^{-2}$ .....	1,100.8	24.5	80.2	4.5	71.1
$3 \times 10^{-2}$ .....	1,651.2	4.5	74.3	17.80	81.2

All of the manganese compounds tested are strong stimulants to the nitrifying organisms. The extent of the stimulation and the quantity of the salt required to produce the maximum stimulation varies greatly with the salt used. The sulphate produces its greatest stimulation at the lowest concentration tested, whereas the chlorid is most active at 8.6 p. p. m. of manganese, the nitrate at 34.4, and the carbonate at 68.8. Manganous chlorid stimulates the nitrifying organisms greater than it does the ammonifying organisms, whereas with the carbonate the reverse is true. It is quite evident from these results that the stimulation exerted by manganese upon soil organisms is governed by the electro-negative ion, which is combined with the manganese and the specific class of organisms on which it is acting.

While manganese is at times added to the soil as a soil amendment, the results reported by different investigators vary, some noting a marked stimulation, while in other experiments it is not so pronounced. The data herein reported, together with those previously reported for ammonification, offer a very plausible explanation of the lack of agreement among various reported experiments.

If we admit that much of the beneficial effect of the manganese on the plant is due to its stimulating influence on the bacterial activity of the soil, thus liberating more available nitrogen, forming organic acids and carbon dioxide, which in turn liberate phosphorus and other elements essential to plant growth, we can readily see that its influence upon a plant growing in a soil well supplied with available nitrogen and phosphorus would not be great. But if the soil contained unavailable plant food, the increased bacterial activity would make more plant food available. This would then be taken up by the growing plant and shown in the increased crop yield.

The manganous carbonate is peculiar in that at the lower concentration it is toxic, but at a concentration of  $125 \times 10^{-6}$  mole it acts as a stimulant. The chlorid is most active at  $156 \times 10^{-7}$  mole, and in the next concentration the nitric nitrogen content of the soil is reduced to one-half-normal, and at a concentration of  $3 \times 10^{-3}$  mole there is only 4.5 per cent of the normal nitric nitrogen present in the soil.

The manganous sulphate stimulates throughout a much wider range of concentrations than does the chlorid, and at the highest concentration tested it had reduced the nitrification to only three-fourths-normal.

Manganous nitrate is a more powerful stimulant than any of the other salts, and stimulates in the same concentrations as does the sulphate, but it is much more toxic in the higher concentrations than are any of the other salts. Although the carbonate is not a very active stimulant at any of the concentrations tested, neither is it very toxic.

#### INFLUENCE OF IRON SALTS

The compounds used in this series were ferric chlorid, ferric sulphate, ferric nitrate, and ferric carbonate. All except the carbonate were added to the soil in solution. The carbonate was added in the form of a dry powder and carefully mixed with the soil. Considerable difficulty was experienced in getting duplicate determinations to agree when the sulphate was applied to the soil, and the results as reported represent the average of eight sets of determinations. The chlorid, nitrate, and carbonate represent the average of four closely agreeing sets of determinations. The results are given in Table IX.

TABLE IX.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of iron salts

[The untreated soil is taken as 100 per cent]

Amount of iron, p. m.	Percentage of nitric-nitrogen formed in presence of			
	Ferric chlorid	Ferric sulphate	Ferric nitrate	Ferric carbonate
None	100.0	100.0	100.0	100.0
2.0	103.4	102.0	95.6	102.5
5.8	101.0	94.2	95.4	104.1
11.6	115.5	67.0	92.4	102.9
13.2	116.5	81.8	90.1	96.0
46.5	118.4	97.1	89.7	99.4
93.0	103.4	97.5	86.2	100.7
186.0	102.6	98.9	89.2	105.0
372.3	78.5	100.0	53.4	110.7
744.6	32.6	84.3	12.4	117.4
1,116.9	10.9	87.9	7.9	104.5

From these results it may be seen that all of the iron salts, with the exception of the nitrates, increase the nitric nitrogen of the soil. The maximum stimulation for the chlorid occurs when 46.5 p. m. of iron

had been applied to the soil, the sulphate at 2.9 p. p. m., and the carbonate at 744.6 p. p. m. The chlorid is a much more powerful stimulant than are any of the other salts, and in this respect there is a marked similarity between the ammonifying and nitrifying organisms.

The results offer a very likely explanation of why there is an increased yield obtained when iron compounds are applied to the soil, as the stimulation of the soil organisms would greatly increase the available plant food. There would be not only more available nitrogen but the increased bacterial activity would render soluble more potassium and especially more phosphorus; the results reported by Griffiths (5) indicate that the plants growing on soil manured with iron sulphate contain more phosphorus than those growing on unmanured soil. We would have to assume either that the application of iron to the soil stimulates a plant so that it requires more phosphorus or else that the iron compounds increase the availability of the phosphorus, and, hence, the plant takes up more. This latter explanation seems the more reasonable, but here we have to look for an indirect effect, for the iron directly depresses the solubility of phosphorus (2).

The ferric nitrate becomes toxic to the nitrifying organisms at a much lower concentration than any of the other iron salts. Furthermore, its toxicity increases much more rapidly than that of any of the other compounds. The chlorid does not become toxic until 372.3 p. p. m. of the iron has been added to the soil, whereas the sulphate is toxic at 5.8 p. p. m. The carbonate was toxic at none of the concentrations tested.

The highest concentration used 1,116.9 p. p. m. of iron in the form of chlorid, reduces the nitric nitrogen content of the soil to 10.9 per cent, the sulphate to 87.9, the nitrate to 7.9, whereas the carbonate containing soil contains 104.5 per cent of normal.

#### INFLUENCE OF CHLORIDS

So far in this discussion we have been comparing the action of compounds having the same electro-positive but a varying electro-negative ion. Hence, the results considered have given us an insight into the influence of the anions Cl, SO<sub>4</sub>, NO<sub>3</sub>, and CO<sub>3</sub> upon the nitrifying efficiency of the soil. It is therefore important that the compounds be compared where the anion is a constant and the cation a variable. This is done in Table X. In this series we have the chlorids of sodium, potassium, magnesium, calcium, manganese, and iron. The experiment was so arranged that equivalent quantities of chlorin in the various forms were applied in 100 gm. of soil. Each reported result is the average of four or more closely agreeing determinations.

TABLE X.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and kinds of chlorids

[The untreated soil is taken as 100 per cent]

Amount of chlorid	Percentage of nitric nitrogen formed in presence of—					
	Sodium chlorid.	Potassium chlorid.	Magnesium chlorid.	Calcium chlorid.	Manganous chlorid.	Ferric chlorid.
None	100.0	100.0	100.0	100.0	100.0	100.0
5.54	102.4	93.5	67.7	87.9	100.6	103.4
11.08	102.4	86.8	58.0	79.0	112.9	101.0
22.16	100.6	106.5	68.5	88.2	57.7	115.5
44.32	103.1	102.3	55.0	84.0	47.7	116.5
88.65	114.7	84.3	97.1	86.8	31.9	128.4
117.30	139.6	84.3	123.2	127.4	44.2	103.4
354.60	142.0	84.8	84.5	160.3	55.2	102.6
709.20	136.2	78.0	78.6	167.6	55.2	78.5
1,418.39	57.5	35.6	26.5	124.5	24.5	32.6
2,127.59	16.4	5.2	11.8	99.2	4.5	10.9

All of the chlorids tested increase the accumulation of nitric nitrogen in the soil, and it would appear from the results that the extent of stimulation is governed largely by the cation while the toxicity of the compound is determined by the anion. Measured in terms of the effect upon nitrification, calcium chlorid is the most effective stimulant of the chlorids tested, followed in the order: Sodium chlorid, ferric chlorid, magnesium chlorid, manganous chlorid, and potassium chlorid.

These results undoubtedly account for the varying results noted when sodium chlorid is used as a fertilizer. Some experimenters obtain a good yield from its use; others obtain just as good a yield without it.

Storp (10), in an article on sodium chlorid as a manure, attributes the benefit derived from its use as being due to the decomposing of insoluble plant food by the sodium chlorid. If this be the correct theory, we can account for yields such as those obtained by Voelcker (11). As an average of five experiments, on land which had been manured with sodium chlorid, the yield of mangels was 36,060 pounds. On the adjoining unmanured ground there were but 26,035 pounds, a difference of a little over 10,000 pounds due to the use of sodium chlorid. Now, if the land was rich in insoluble plant food and the chlorid was able to liberate it, we could expect a large yield. On the other hand, if the land had been poor in unavailable plant food, no good result would have followed its use. Wheeler (12) seems to have established the fact that sodium chlorid can not to any great extent take the place of potassium salts. However, he does think that sodium chlorid can liberate phosphorus from insoluble forms, as the following statement will show:

It may, however, be stated here that sodium salts seem to liberate phosphorus and potassium so that under certain circumstances they may act as an indirect manure.

In a later report (13, p. 196-202) he shows that the percentage of phosphorus in a plant is increased by the use of a sodium salt. With radish this was in some cases as much as 0.052 per cent more in the crop from land which had received a full ration of sodium over that which received but a part ration. In the case of turnips there was a difference of 0.121 per cent, the beets 0.035 per cent, the carrots 0.074 per cent, while in the case of the chicory the results are practically the same in the crop from the manured and unmanured land. The report contains many more cases in which the sodium salt increased the phosphorus in the plant. However, the laboratory tests which have been made on phosphates show that sodium chlorid depresses the solubility of a phosphate (2).

It is therefore evident from the results obtained that sodium chlorid increases the ammonia and nitric-nitrogen accumulation of the soil, and all indications point to the conclusion that this is due to an increased activity of bacteria, which bring about this transformation. This being the case, there must be an increase of the nitrites produced in the soil by nitrosomonas, and Hopkins and Whiting (7) have demonstrated that these organisms possess the power of rendering soluble the phosphorus of the soil. Therefore we could expect to find an increased yield when sodium chlorid is added to a soil deficient in soluble phosphate but containing considerable insoluble phosphate, this increase being due to the liberation of phosphorus, which we find revealing itself in a greater phosphorus content of the plants, as noted in the above references.

The concentration at which the various compounds are found to exert their greatest stimulating action varies greatly with the compound. The maximum for the different compounds is as follows: Calcium chlorid 709.2 p. p. m., chlorin as sodium chlorid 354.6 p. p. m., chlorin as ferric chlorid 88.7 p. p. m., chlorin as magnesium chlorid 117.3 p. p. m., chlorin as manganous chlorid 11.1 p. p. m., chlorin as potassium chlorid 22.2 p. p. m.

The point at which the specific compound becomes toxic varies greatly with the cation. With the exception of calcium chlorid there is nearly the same quantity of nitric nitrogen in all soils at the highest concentration, 2,127.6 p. p. m. of chlorin in the various combinations.

The difference in toxicity of the compounds becomes greater when we compare equal molecular portions of the different compounds, as may be seen from Table XI.



TABLE XI.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and to which were added varying amounts and kinds of chlorids in equal molecular proportions

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Percentage of nitric-nitrogen formed in the presence of—				
	Sodium chlorid.	Potassium chlorid.	Magnesium chlorid.	Calcium chlorid.	Manganous chlorid.
None.....	100.0	100.0	100.0	100.0	100.0
$150 \times 10^{-7}$ .....	102.4	93.5	58.0	79.0	112.9
$312 \times 10^{-7}$ .....	102.5	86.8	68.5	88.2	57.7
$625 \times 10^{-7}$ .....	100.6	106.5	55.0	84.0	41.7
$125 \times 10^{-6}$ .....	103.1	102.3	97.1	86.8	31.9
$25 \times 10^{-5}$ .....	114.7	84.3	123.2	127.4	44.2
$5 \times 10^{-4}$ .....	139.6	84.3	84.5	160.3	55.2
$1 \times 10^{-3}$ .....	142.0	84.8	88.6	167.6	55.2
$2 \times 10^{-3}$ .....	136.2	78.0	26.5	124.5	24.5
$4 \times 10^{-3}$ .....	57.5	36.6	11.8	99.2	4.5

This would be taken to indicate that the toxicity of the salt is due to physiological action upon the bacteria and not the osmotic pressure exerted by the several compounds.

#### INFLUENCE OF SULPHATES

The compounds used in this series were the sulphates of potassium, sodium, calcium, magnesium, manganese, and iron. The quantity of the salt used in each case was such that equivalent quantities of sulphate in the various forms were added to 100 gm. of soil. It was also of such a concentration, with the exception of the iron sulphate, that equal molecular proportions of the various salts were added to 100 gm. of soil. The results, as averages of a number of closely agreeing determinations, are given in Table XII.

TABLE XII. Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of sulphates

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Amount of sulphate.	Percentage of nitric nitrogen formed in presence of—					
		Sodium sulphate.	Potassium sulphate.	Calcium sulphate.	Magnesium sulphate.	Manganous sulphate.	Ferric sulphate.
None.....	None.	100.0	100.0	100.0	100.0	100.0	100.0
$78 \times 10^{-7}$ .....	7.5	87.8	79.7	117.1	95.2	113.2	102.0
$150 \times 10^{-7}$ .....	15.0	60.2	95.7	115.9	96.2	100.0	94.2
$312 \times 10^{-7}$ .....	30.0	57.1	95.0	143.0	101.2	106.6	67.0
$625 \times 10^{-7}$ .....	60.0	86.2	93.8	140.9	87.1	106.6	82.8
$125 \times 10^{-6}$ .....	120.1	74.0	73.4	125.0	88.7	104.1	97.1
$25 \times 10^{-5}$ .....	240.2	55.1	70.4	148.8	94.6	97.6	97.5
$5 \times 10^{-4}$ .....	480.3	65	67.2	151.7	90.4	101.3	98.9
$1 \times 10^{-3}$ .....	960.6	63.2	62.3	180.6	82.9	88.7	100.0
$2 \times 10^{-3}$ .....	1,921.2	63.0	51.5	196.7	63.3	80.2	84.3
$3 \times 10^{-3}$ .....	2,881.8	50.8	32.4	189.2	59.6	74.3	87.9

Sodium sulphate and potassium sulphate are the only sulphates which fail to increase the nitric-nitrogen content of the soil, and in this respect they are similar to the ammonifiers (3). The degree and concentration at which the various salts stimulate vary with the compound, being greatest with calcium sulphate and least with magnesium sulphate.

Calcium sulphate is the most powerful soil stimulant known. This is due not to a direct nutritive value but to the liberation of plant food which may in a measure be due to the direct interchange between calcium and potassium. However, these results clearly indicate that its main influence is upon the bacterial activities of the soil, especially the ammonifying and nitrifying organisms of the soil. In this manner the available nitrogen of the soil is increased. Furthermore, in the metabolic processes of these bacteria there are formed acids and other compounds which act as solvents for the potassium and phosphorus of the soil. It is well known that the addition of gypsum increases the potassium of the crop, and some other experiments show that it increases the phosphorus, for example, Boussingault (9, v. 1, p. 327) found a greater amount of phosphorus in land manured with gypsum. The phosphorus in the clover from the manured land was 10.57 kilos; that from the unmanured 4.80 kilos. The following year, although no more manure was applied, the phosphorus from the hay grown on the manured land was 6.93 kilos more than from the unmanured.

Although magnesium, manganous, and iron sulphate all increase at some concentration the nitric nitrogen of the soil, they are not nearly as active as is calcium sulphate.

The toxicity of sodium and potassium sulphate is very marked even at the lowest concentrations tested, and increase with increasing quantities of the salt. Although the toxicity of potassium sulphate does not increase as rapidly at the lower concentrations as does that of sodium sulphate, yet at the highest concentration tested,  $3 \times 10^{-3}$  mole, the sodium-sulphate-treated soil contains 50.8 per cent normal of nitric nitrogen, whereas that of the potassium sulphate treated soil contains 32.4 per cent.

Calcium sulphate is not toxic at any of the concentrations tested, whereas magnesium sulphate probably first becomes toxic at a concentration of  $628 \times 10^{-7}$  mole, and at  $3 \times 10^{-3}$  mole the nitric nitrogen content of the soil had been reduced to about one-half normal.

Manganous sulphate and iron sulphate are similar in that they are only slightly toxic even at the higher concentrations. It is evident from these results that the stimulating action is due mainly to the electro-positive ion, whereas the electro-negative ion determines the toxicity of the compound.

## INFLUENCE OF NITRATES

The compounds used in this series were sodium nitrate, potassium nitrate, calcium nitrate, magnesium nitrate, manganous nitrate, and ferric nitrate. The quantity added to the soil was such that in each case equivalent quantities of nitrate in the various forms were added to the soil. Hence, the varying factor is the electro-positive ion, the electro-negative remaining the same in each case. The average results for a number of closely agreeing determinations are given in Table XIII as percentages of nitric nitrogen found in 100 gm. of soil, the untreated soil being taken as 100 per cent.

TABLE XIII. *Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of nitrate salts*

[The untreated soil is considered as 100 per cent]

Amount of nitrate.	Percentage of nitric nitrogen formed in presence of —					
	Sodium nitrate.	Potassium nitrate.	Calcium nitrate.	Magnesium nitrate.	Manganous nitrate.	Ferric nitrate.
<i>P. p. m.</i>						
None	100.0	100.0	100.0	100.0	100.0	100.0
9.7	92.7	106.4	99.1	92.9	121.3	95.6
19.4	88.3	74.7	88.4	85.7	113.8	95.4
38.8	94.4	98.6	92.5	106.5	113.5	92.4
77.6	101.	92.5	102.1	87.	125.4	90.1
155.2	75.9	100.5	92.1	95.7	107.9	89.7
310.4	71.6	71.1	100.8	65.2	79.4	86.2
624.8	69.4	33.5	86.9	43.5	49.2	89.2
1,241.6	48.0	26.0	46.8	—	26.9	53.4
2,483.2	17.1	2.5	—	5.0	4.5	12.4
3,724.8	—	—	—	—	—	—

Sodium nitrate does not stimulate the nitrifying organisms in any of the concentrations, whereas potassium in the lowest concentration does. Otherwise these two compounds are very similar in action. Both in low concentrations depress the accumulation of nitric nitrogen in the soil, but at a higher concentration this toxicity disappears. When the quantity of nitrates added to the soil exceeds 310.4 p. p. m., the nitric nitrogen rapidly disappears from the soil. At the highest concentrations tested the added nitric nitrogen was rapidly disappearing.

Calcium and magnesium nitrate in the lower concentrations are quite similar in action, but at 38.8 p. p. m. of nitric nitrogen in the form of magnesium nitrate is more active as a soil stimulant than the calcium in any of the concentrations tested. At the higher concentrations the nitric nitrogen disappeared from the soil more rapidly where the magnesium salt was added than where the calcium salt was added.

Manganous nitrate is the strongest nitrate stimulant tested, at one concentration increasing the nitric-nitrogen content of the soil one-

fourth. But even in the presence of large quantities of this salt there is a notable disappearance of nitric nitrogen from the soil.

Ferric nitrate is peculiar, in that it fails to stimulate in any of the concentrations tested, and there is a gradual increase in toxicity from concentration to concentration. However, even where the largest quantity of iron nitrate was added, there was no loss of nitric nitrogen from the soil.

The occurrence of the negative results where the sodium, potassium, calcium, magnesium, and manganous nitrate are added to the soil raises the question, Has denitrification taken place or does it stimulate other bacterial activities so that the nitric nitrogen is rapidly transformed into protein nitrogen?

In order to answer this question, a set was prepared in which the various salts were added to the soil and incubated for 21 days, and the total nitrogen determined. The results are given in Table XIV. Each is the average of four determinations.

TABLE XIV.—Quantity of nitrogen obtained from 100 gm. of soil receiving various treatments

Treatment.	Nitrogen in 100 gm. of soil.	Excess in nitrate- treated soil	Gain or loss in nitrogen over soil re- ceiving no nitrate.
	<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
Dried blood, no nitrate.....	333.9		
Dried blood, 84.06 mgm. of nitric nitrogen as sodium nitrate.....	419.3	85.4	1.34
Dried blood, 84.06 mgm. of nitric nitrogen as calcium nitrate.....	460.5	126.6	42.54
Dried blood, 84.06 mgm. of nitric nitrogen as ferric nitrate.....	455.0	121.1	37.04
Dried blood, 84.06 mgm. of nitric nitrogen as magnesium nitrate.....	466.5	132.6	48.54
Dried blood, 84.06 mgm. of nitric nitrogen as manganous nitrate.....	443.1	109.2	25.14
Dried blood, 84.06 mgm. of nitric nitrogen as potassium nitrate.....	412.0	78.1	5.96

It is evident from these results that the loss of nitric nitrogen from this soil is not due to denitrification, for we find in every case, with the exception of where the potassium nitrate was added to the soil, that there was a gain of combined nitrogen. This is remarkable, for we have here a soil which was low in nitrogen but to which had been added 2 per cent of dried blood greatly stimulated in its nitrogen-fixing powers. The quantity of nitrogen fixed by this soil on the addition of the various nitrates in some cases is from four to eight times that normally fixed by the soil. Whether the azofiers would continue to fix nitrogen at this speed is doubtful, but experiments are under way in this laboratory to decide this point. If they will, it opens up an interesting and practical field for investigation.

## INFLUENCE OF CARBONATES

The compounds used in this series were the carbonates of sodium, potassium, calcium, magnesium, manganese, and iron. The results as percentages of nitric nitrogen, the untreated soil being considered as 100 per cent, are given in Table XV.

TABLE XV.—Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of carbonates

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil	Amount of carbonate.	Percentage of nitric nitrogen formed in presence of—					
		Sodium carbonate	Potassium carbonate	Calcium carbonate	Magnesium carbonate	Manganese carbonate	Ferric carbonate
None	P. p. m.	100.0	100.0	100.0	100.0	100.0	100.0
78×10 <sup>-1</sup>	None.	100.0	100.0	100.0	100.0	100.0	100.0
78×10 <sup>-1</sup>	4.7	100.0	99.1	99.4	70.4	91.2	102.5
156×10 <sup>-1</sup>	9.4	79.4	92.7	97.4	97.7	72.2	104.1
312×10 <sup>-1</sup>	18.7	76.9	81.3	97.2	108.1	77.8	102.9
625×10 <sup>-1</sup>	37.5	88.2	80.3	85.3	136.1	86.3	96.0
125×10 <sup>-2</sup>	75.0	94.1	69.0	95.9	119.8	108.4	99.4
25×10 <sup>-3</sup>	150.0	79.4	71.0	97.2	140.7	86.8	100.7
5×10 <sup>-4</sup>	300.0	73.5	63.7	79.0	101.2	84.6	105.6
1×10 <sup>-3</sup>	600.0	70.5	55.4	82.0	116.0	84.6	110.7
2×10 <sup>-3</sup>	1,200.0	61.5	41.8	59.2	72.7	71.1	117.4
3×10 <sup>-3</sup>	1,800.0	58.8	14.8	69.1	51.7	81.2	104.5

Neither sodium nor potassium carbonate stimulate in any of the concentrations tested, and in this respect they differ sharply from their action on the ammonifiers. Both compounds become toxic at a concentration of  $156 \times 10^{-1}$  mole, but the toxicity of the potassium salt increases much more rapidly than the toxicity of the sodium salt. The latter, at a concentration of  $3 \times 10^{-3}$  mole, reduces the nitric-nitrogen content of the soil to 58.8, while the former, at the same concentration, reduces it to 14.8 per cent.

Calcium carbonate gradually increases in toxicity from the lower to the higher concentrations, whereas the magnesium carbonate, up to a concentration of  $1 \times 10^{-3}$  mole, is a strong stimulant. Although the calcium is toxic at the lowest concentration, and although magnesium stimulates, yet at the highest concentration tests,  $3 \times 10^{-3}$  mole, there is considerable more nitric nitrogen in the calcium-carbonate-treated soil than in the magnesium-carbonate-treated soil.

Manganese stimulates slightly when  $125 \times 10^{-2}$  mole are added to the soil, above which it becomes slightly toxic, next to iron carbonate; manganese carbonate is the least toxic of the carbonates tested. Iron carbonate is the most powerful of the stimulants tested and becomes toxic only in two concentrations,  $625 \times 10^{-1}$  mole and  $125 \times 10^{-2}$  mole. Above and below these concentrations it is a stimulant.

## RELATION BETWEEN BACTERIAL ACTIVITY AND HIGHER PLANTS

The results herein reported, together with those published by Dr. Harris (6), make it possible to compare the influence of some of the salts upon the nitrifying powers of a soil with their influence upon the higher plants. This comparison is quite justifiable, for the same soil was used in the experiments with seedlings as has been used in the work on bacterial activities. The comparison is made in Table XVI. The results, as reported, are the quantities of the respective salts which are necessary to reduce nitrification and the production of dry matter in wheat seedlings to about half-normal.

TABLE XVI.—Percentage of various salts in loam soil necessary to reduce nitrification, germination, and dry matter product in wheat to about half-normal

Salt.	Reduction of nitrification to about half-normal	Reduction of wheat seedling to about half-normal	Excess required by bacteria
Magnesium chlorid.....	0.006	0.40	0.394
Magnesium nitrate.....	.074	.45	.376
Potassium nitrate.....	.101	.40	.299
Potassium carbonate.....	.138	.70	.562
Sodium nitrate.....	.170	.30	.130
Sodium carbonate.....	.212	.30	.088
Sodium chlorid.....	.234	.20	.034
Potassium chlorid.....	.298	.25	.048
Potassium sulphate.....	.349	.60	.251
Magnesium sulphate.....	.361	.70	.339
Sodium sulphate.....	.568	.55	.013

Four of the salts tested, sodium chlorid, potassium chlorid, sodium sulphate, and calcium chlorid, are less toxic to the nitrifying organisms than they are to wheat seedlings. All of the other compounds are much more injurious to nitrifying bacteria than they are to wheat seedlings. In many cases the wheat seedlings will withstand many times as much of the salt as will the plant. This is especially noticeable in the case of magnesium nitrate and magnesium chlorid. The plant being able to withstand 70 times as much of this latter compound as will bacteria.

It is certain from these results that a test of the ammonifying power of an alkali soil gives a better index of its crop-producing power than does a determination of its nitrifying power, for a close correlation was found to exist between the toxicity of many of these salts for ammonifying organisms and wheat seedlings (3).

## RELATIVE STIMULATION OF THE VARIOUS SALTS

It has been noted repeatedly throughout this work that many of the salts tested increase the accumulation of nitric nitrogen in the soil. The extent of this stimulation and the concentration of the specific salts re-

quired to produce the maximum effect vary greatly with the salt. These facts are summarized in figures 1 and 2.

Only 6 of the compounds tested, sodium sulphate, sodium carbonate, potassium sulphate, potassium carbonate, calcium carbonate, and ferric nitrate, failed to increase the nitric-nitrogen content of the soil. The 18 others all increased the nitric-nitrogen content of the soil. There is no correlation between the stimulation of the ammonifying and nitrifying processes of the soil. This is remarkable when we remember that the speed of the latter is undoubtedly controlled and dependent upon the other. And the results herein reported probably indicate that there are other side reactions taking place which are influenced by these salts but which are not measured by these methods.

Averaging the molecular weights for the 12 compounds acting as the strongest stimulants, we find them to be considerably lower than the average molecular weight of those which exert little stimulating influence. We really find some of the compounds with the lowest molecular weight—for instance, sodium chlorid—the greatest stimulants. Hence, it would seem that Grutzer's generalization for animal stimulants does not hold for either the ammonifying or nitrifying organisms. Some of the strongest stimulants for plants, sodium chlorid and calcium sulphate, increase to the greatest extent the nitric-nitrogen content of the soil. Therefore it is certain that the increased plant growth is due to a

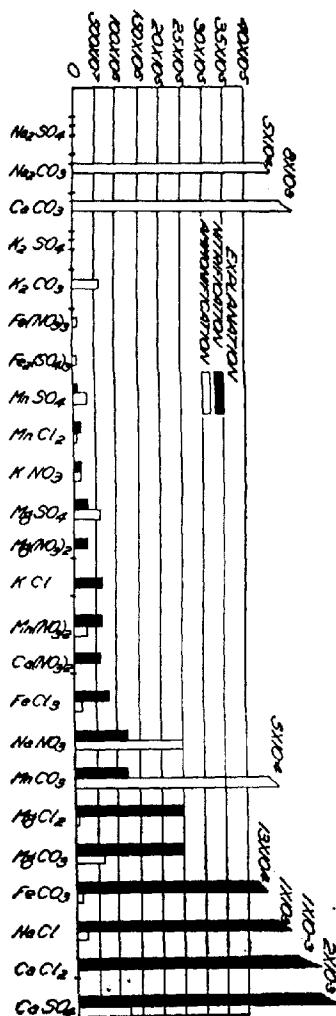


FIG. 1.—Graphs showing molecular concentrations at which the highest stimulation is noted

phosphate, increase to the greatest extent the nitric-nitrogen content of the soil. Therefore it is certain that the increased plant growth is due to a

great extent to the increased available plant food yielded by the accelerated bacterial activity of the soil.

The quantity of the salt necessary to produce maximum stimulation varies greatly with the salt. It is usually the case that those compounds which are the greatest soil stimulants must be added in larger quantities to produce maximum stimulation than those which are not as active stimulants and which produce their greatest effect at lower concentrations.

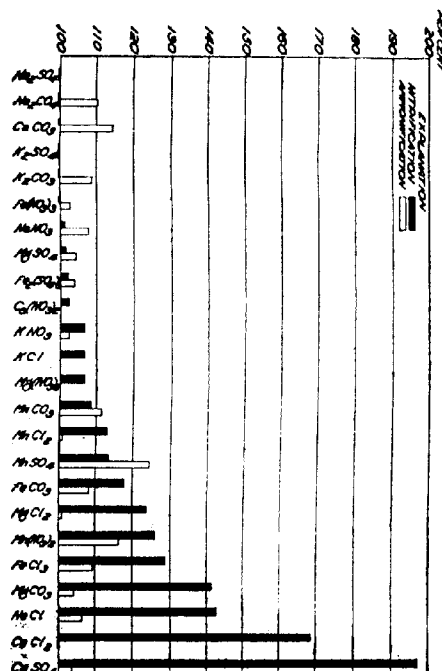


FIG. 2.—Graphs showing the percentage of stimulation at the above noted molecular concentrations (see fig. 1), the untreated soil being counted as producing 100 per cent of nitric nitrogen.

#### RELATIVE TOXICITY OF THE VARIOUS SALTS

The salts used in this work may be compared as to toxicity from three viewpoints: First, the lowest concentration of the salt at which a toxic effect is noted toward the nitrifying organisms; second, the molecular concentration at which nitric-nitrogen accumulation is reduced to three-fourths normal; and third, the percentage of nitric-nitrogen produced in the presence of the largest quantity of the various salts, which is  $2 \times 10^{-3}$  mole of the salt in 100 gm. of soil. These results are reported in figures 3,



4, and 5. Not one of the compounds tested was toxic at the lowest concentration tested,  $78 \times 10^{-7}$  mole. All of the others became toxic at some

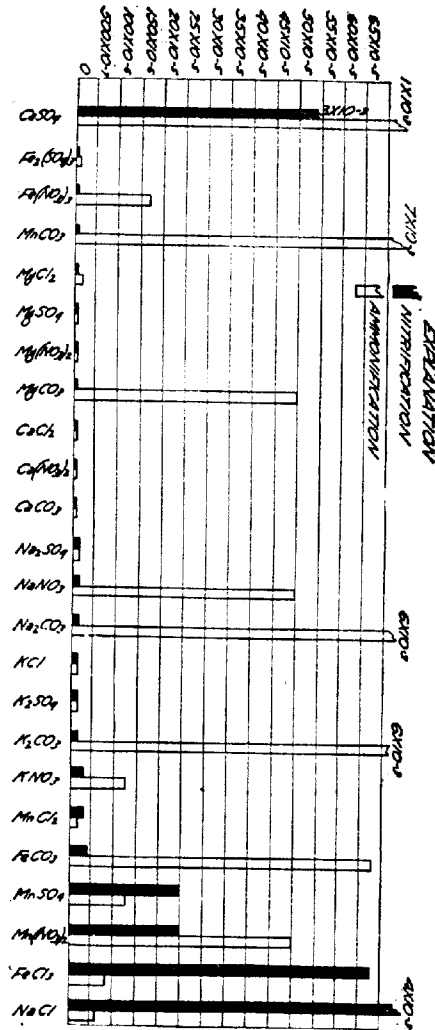


FIG. 3.—Graphs showing the molecular concentrations at which the various salts are toxic to nitrification

of the concentrations tested. In 11 out of the 20 cases tested the point of toxicity for the ammonifiers and nitrifiers were the same, whereas

in the remaining cases the quantity required to become toxic to the ammonifiers was much greater than it was for the nitrifiers. In only

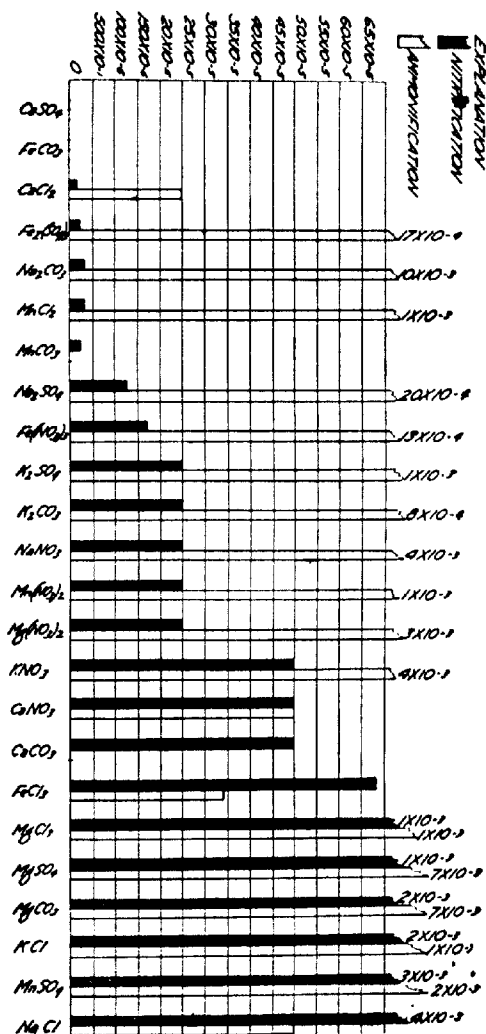


FIG. 4.—Graphs showing the molecular concentrations which reduce the nitrification to three-fourths normal

three instances were the salts more toxic to ammonifiers than to nitrifiers.

It is evident from these results that while the increased osmotic pressure exerted by the salts added to a soil plays an important part in the retarding of the bacterial activity, it is not the only factor nor probably the main one. The principal factor is probably a physiological one caused by the action of the substance upon the living protoplasm of the cell, changing its chemical and physical properties so that it can not function normally. However, we do not find a relationship between the toxicity of the compound and its power to precipitate colloids. It appears, therefore, that while the precipitation of the colloidal cellular

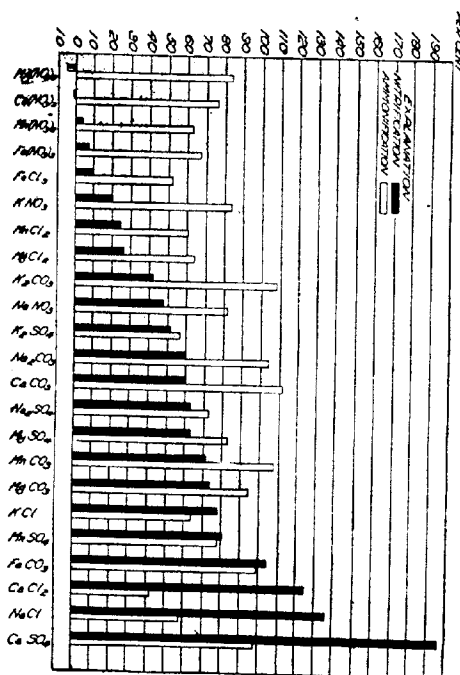


FIG. 5.—Graphs showing the percentages of nitric nitrogen produced in 100 gm. of soil to which had been added  $2 \times 10^{-4}$  mole of the various salts, the untreated soil being counted as producing 100 per cent.

material often causes death of the organisms, it is not necessarily the determining factor in the toxic action of these salts.

As can be seen from figure 4, it is not necessarily those compounds which become toxic at the lowest concentration which have the greatest far-reaching effect upon the bacterial activities of the soil. This condition holds for both the ammonifying and nitrifying organisms. It requires in almost every case more of the specific salt to reduce ammonifi-

cation to three-fourths normal than is required to produce the same effect upon the nitrifiers. It is evident from these results that the common soil alkalis, calcium chlorid, sodium carbonate, sodium sulphate, and sodium nitrate, are very toxic to nitrifying organisms, and if present to any great extent, will greatly reduce the nitric-nitrogen content of the soil.

The toxicity of the compound to ammonification was found to be controlled largely by the cation, but no such a relationship is found to exist in the case of the nitrifiers, as can be seen from figure 5.

The toxicity of the compounds to ammonification was found to be controlled largely by the cation, but no such relationship is found to exist in the case of the nitrifiers (fig. 5).

#### SUMMARY

The toxicity of the chlorids, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron as determined by nitrification is determined by the specific salt and not by the electro-negative ion, as was the case with the ammonifiers. With the exceptions of the manganous chlorid and sulphate and the chlorids of iron and sodium, the salts tested all became toxic at a lower concentration to the nitrifiers than to the ammonifiers.

The quantity of a salt which can be applied to a soil without decreasing the nitric-nitrogen accumulation in the soil varies with the salt, and for the soil under investigation it is in the order of decreasing toxicity of the salts as follows: Sodium sulphate, sodium carbonate, calcium carbonate, potassium sulphate, potassium carbonate, ferric nitrate, sodium nitrate, magnesium sulphate, ferric sulphate, calcium nitrate, potassium nitrate, potassium chlorid, magnesium nitrate, manganous carbonate, manganous chlorid, manganous sulphate, ferric carbonate, magnesium chlorid, manganous nitrate, ferric chlorid, magnesium carbonate, sodium chlorid, calcium chlorid, and calcium sulphate.

It is not necessarily those compounds which become toxic in the lowest concentrations which are most toxic in higher concentrations, as the toxicity of some salts increase more rapidly than the toxicity of others.

It is quite evident from the results reported that the increased osmotic pressure exerted by the salt added to the soil plays a minor part in the retarding of the bacterial activity. The main factor is probably a physiological one due to the action of the substance upon the living protoplasm of the cell, changing its chemical and physical properties so that it can not function normally.

The common soil "alkalis," calcium chlorid, sodium sulphate, sodium carbonate, and the less common one, calcium nitrate, are very toxic to the nitrifying organisms, and if present in soil to any great extent will greatly reduce the nitric-nitrogen accumulation in such a soil.

Sodium sulphate, sodium carbonate, calcium carbonate, potassium sulphate, potassium carbonate, and iron nitrate failed to increase the nitric-nitrogen accumulation in a soil. All of the others, however, in some of the concentrations tested acted as stimulants. The extent of the stimulation and quantity of salt necessary for maximum stimulation varied with the specific compound. Naming them in the order of increasing efficiency, they are: Sodium nitrate, magnesium sulphate, ferric sulphate, calcium nitrate, potassium nitrate, potassium chlorid, magnesium nitrate, manganous carbonate, manganous chlorid, manganous sulphate, ferric carbonate, magnesium chlorid, manganous nitrate, ferric chlorid, magnesium carbonate, sodium chlorid, calcium chlorid, and calcium sulphate. The last two increased the nitric-nitrogen accumulation of the soil 67 and 97 per cent, respectively.

Those compounds which are the strongest plant stimulants are also the most active in increasing the nitric-nitrogen accumulation of the soil and it is very likely that the effect upon the plant is due mainly to the action of the compound upon the bacteria which in turn render available more plant food.

Many of the nitrates caused large losses of nitric nitrogen from the soil; this is due to the stimulation of other species which transform the nitric nitrogen into protein nitrogen and not to denitrification.

Magnesium nitrate, ferric nitrate, calcium nitrate, and manganous nitrate are very active stimulants of the nitrogen-fixing organisms. In some cases these compounds increased nitrogen fixation many times over that in the normal soil.

The ammonifying powers of a soil containing alkalis are a better index of its crop-producing powers than are the nitrifying powers.

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